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Composite materials based on silk proteins

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Abstract

A number of animals have evolved to produce silk-based composite materials for a variety of task-specific applications. The review initially focuses on the composite structure of silk fibers produced naturally by silkworms and spiders, followed by the preparation and applications of man-made composite materials (including fibers, films, foams, gels and particulates) incorporating silk proteins in combination with other polymers (both natural and synthetic) and/or inorganic particles.

Keywords

- Biomimetic;
 - Biomaterials;
 - Composite;
 - Proteins;
 - Silk
-

1. Introduction

A number of animals (best known being arthropods) have evolved to produce a variety of task-specific silk protein-based composite materials (see [Table 1](#)) [\[1\]](#). Of the greatest economic importance are *Bombyx mori* (*B. mori*) silkworms that produce cocoons from silk composite fibers to protect them from predators during their metamorphosis into moths. Web-weaving spiders (such as *Araneus diadematus* or *Nephila clavipes*) produce a number of different silk composite fibers to capture prey (in webs), to protect/preserve their offspring/prey (in cocoons), and as lifelines to escape from predators. Certain silk fibers have mechanical properties superior to Nylon, Kevlar and high-tensile steel. The mechanical properties and biocompatibility of naturally occurring silkworm and spider silk composite fibers have allowed humans to use such fibers for millennia for applications as diverse as currency, hunting (bow strings, cross-hairs, fishing lines or nets), paper, textiles and wound dressings [\[2\]](#), [\[3\]](#) and [\[4\]](#).

Table 1.

Examples of natural composite materials incorporating silk proteins.

Example	Components	Role	References
Silkworm cocoons	Fibroins	Mechanical properties	[11] , [12] , [13] , [14] , [15] , [16] , [17] and [18]
	Sericins	Glue-like glycoproteins	
	Peptides	Antimicrobial	
	Lipids	Camouflage	
Spider webs (e.g. lifeline)	Major ampullate spidroins	Mechanical properties	[6] , [7] , [19] , [20] , [21] , [22] , [23] , [24] and [25]
	Minor ampullate-like spidroin	Mechanical properties	
	Glycoproteins	Unknown	
	Lipids	Unknown	
Mussel byssus fibers	Collagens with specific flanks:	Mechanical properties	[8] , [9] and [10]
	preCol-D	Spider silk-like (stiff)	
	preCol-P	Elastin-like (elastic)	
Mollusc shells	Silk-like protein	Energy distribution	[140] , [166] , [167] and [168]
		Mechanics	
	Chitin	Scaffold	
	Calcium carbonate	Hard mineral	

[Table options](#)

This article begins with a brief explanation of the composite structure and function of natural fibers based on silk proteins [\[5\]](#), [\[6\]](#), [\[7\]](#), [\[8\]](#), [\[9\]](#) and [\[10\]](#), followed by the preparation of man-made composite materials based on silk proteins and their prospective applications.

1.1. Fibers produced by silkworms are natural composites

Mankind has farmed *B. mori* silkworms for thousands of years, which has facilitated an understanding of its composite structure (see [Table 1](#) and [Fig. 1](#)), and more recently its potential for biomedical applications [\[11\]](#). Silkworm silk fibers are composed of two protein microfilaments (known as brins) embedded in a glue-like glycoprotein coating (see [Fig. 1B](#) and [C](#)) [\[12\]](#). The brins are fibroin filaments composed of bundles (of ca. 100 nm in diameter) of nanofibrils (individually of ca. 5 nm in diameter) that are preferentially aligned with the long axis of the fiber [\[13\]](#) and [\[14\]](#).

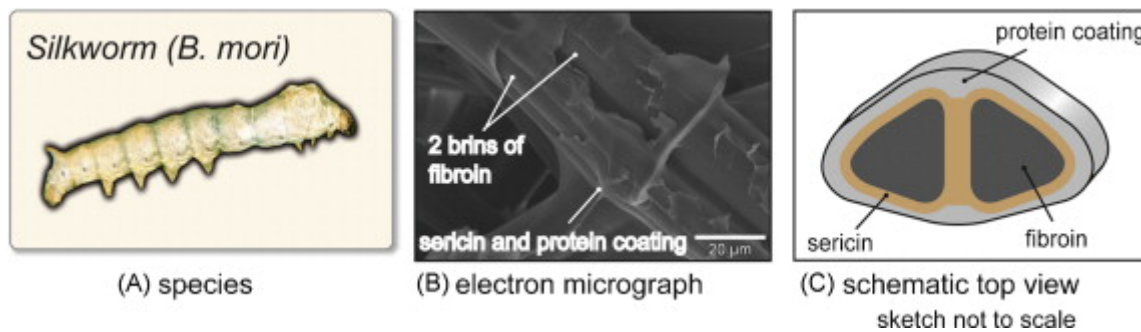


Fig. 1.

A) Photograph of a *Bombyx mori* silkworm. B) Electron micrograph of partially degummed *B. mori* silkworm cocoon fibers, in which the two brins of fibroin and the coating of sericins and other proteins postulated to protect the cocoon against microbes and predators are pointed out. C) Schematic illustration of the composite structure of a cocoon fiber, in which the two brins of fibroin and the coating of sericins and other proteins postulated to protect the cocoon against microbes and predators are pointed out [2]. Adapted with the permission of both the authors and publisher, Copyright 2008, Elsevier Science Ltd., Oxford, UK.

[Figure options](#)

The fibroin nanofibrils are composed of a complex of three proteinaceous components: a large protein, known as heavy chain (H-chain) fibroin (of ca. 350 kDa) that is linked to a second small protein, known as light chain (L-chain) fibroin (of ca. 25 kDa) via disulfide bonds; and a third small glycoprotein, known as the P25 protein (of ca. 30 kDa) is associated via non-covalent hydrophobic interactions. The molar ratios of H-chain:L-chain:P25 are 6:6:1; the H-chain is hydrophobic and contains blocks of (Gly-Ala-Gly-Ala-Gly-Ser)_n that are known to form anisotropic β-sheet-rich nanocrystals, whereas the L-chain is more hydrophilic and relatively elastic, and the P25 protein is believed to play a role in maintaining the integrity of the complex [15].

The glue-like glycoproteins are known as sericins (a set of serine-rich glycoproteins) that coat the fibroin filaments and ensure the cohesion of the cocoon by sticking the twin filaments together and constitute 25–30% of the weight of the fiber (see Fig. 1B and C) [16]. Finally the fiber is coated with a variety of other proteins postulated to protect the cocoon against microbes and predators (see Fig. 1C) [17] and [18].

1.2. Fibers produced by spiders are natural composites

Silk fibers made of proteins produced in the major ampullate (MA) silk gland (of ca. 250–350 kDa) have a very high-tensile strength (comparable to Kevlar) and moderate elasticity. MA silks are used as a scaffold upon which to attach other silks during the construction of a web and as a lifeline when it is necessary to escape from a predator. MA silks have diameters between 1 and 20 μm (depending upon spider species) and have a core-shell type structure (see Fig. 2 and Table 1) [6] and [7]. The core contains two major proteins (e.g. MaSp1 and MaSp2 produced by *N. clavipes* spiders or ADF-3 and ADF-4 produced by *Araneus diadematus* spiders) that are composed predominantly of glycine, alanine and proline (although the quantity of the latter varies significantly between individual proteins and spider species) [19]. Major ampullate spidroins are reminiscent of block copolymers containing

blocks of $(\text{Ala})_n$, $(\text{Gly-Ala})_n$, $(\text{Gly-Gly-X})_n$ or $(\text{Gly-Pro-Gly-X-X})_n$ where X is typically tyrosine, leucine or glutamine. The alanine-rich blocks $(\text{Ala})_n$ and the $(\text{Gly-Ala})_n$ flanking them are known to form β -sheet structures that are responsible for the high-tensile strength of MA silks; whereas the blocks of (Gly-Gly-Ala) form 3_1 -helices, and the blocks of Gly-Pro-Gly-X-X form β -turn spirals imparting elasticity/flexibility to the proteins [20], [21], [22] and [23].

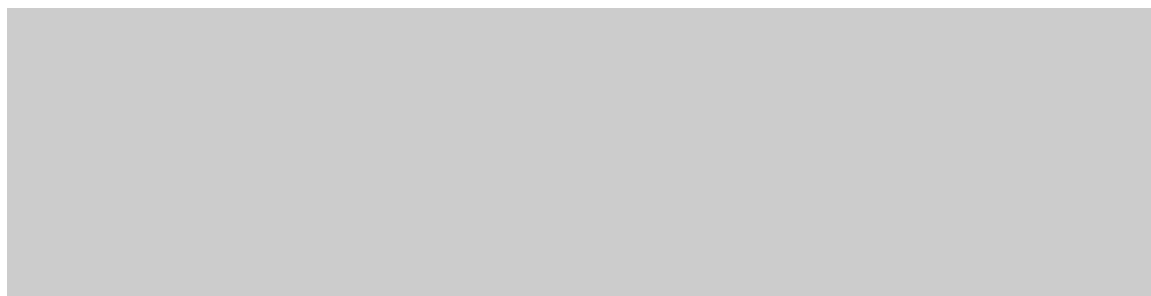


Fig. 2.

A) Photograph of an *Araneus diadematus* spider. B) Electron micrograph of major ampullate fibers. C) Schematic illustration of the composite structure of a major ampullate fiber. The core fiber is composed of major ampullate (MA) spidroins, coated by a layer of minor ampullate-like (MI-like) spidroin known as the skin, a layer of glycoprotein and finally a layer of lipids [2]. Adapted with the permission of both the authors and publisher, Copyright 2008, Elsevier Science Ltd., Oxford, UK.

[Figure options](#)

Of particular note is that the two major proteins comprising the core filament (of fibers spun by *N. clavipes* spiders) are inhomogeneously distributed [6] and [7]. The origin of the inhomogeneous distribution is believed to be due to the difference in primary amino acid sequence of the two proteins [24] and [25]. MaSp1 is distributed more or less homogeneously throughout the core of the lifeline fiber forming a β -sheet crystal reinforced matrix, with elongated island-like structures of MaSp2 in the core of the fiber that are believed to act as deformable shock absorbers, improving the elasticity of the fibers. These shock absorbers further reinforce the fiber, thereby reducing the likelihood of breaking upon the impact of prey into the web. This core filament is coated in a layer of minor ampullate (MI) spidroin-like protein (known as the 'skin'), a glycoprotein coat, and finally a lipid coat (see Fig. 2C) [6] and [7].

1.3. Sources of silk proteins

Although many animals produce silk proteins for various different applications [1], in this review we will focus on the silkworm and spider silk proteins as they are the most extensively studied and readily abundant.

Humans domesticated *B. mori* silkworms thousands of years ago in order to be able to produce silk on an industrial scale for a variety of applications. A simple washing procedure after harvesting the cocoons removes the sericin coating on the silk fibers yielding clean *B. mori* silk fibroin [16]. A similar process is applicable to silk fibers produced by wild silkworms (such as *Antheraea pernyi*, *Antheraea mylitta*, or *Samia cynthia ricini* silkworms [16]) providing us with a source of silk proteins with differing amino acid sequences in the

protein (therefore differing biocompatibility, biodegradability and solubility in various solvents).

Although it is possible to harvest milligrams of spider silk fibers, either at their point of application (e.g. from their egg cocoons or webs) or directly from the animal (via *forced silking*), this is an incredibly time consuming and expensive process (e.g. a 3.4 m rug produced from the major ampullate silk fibers of *Nephila madagascarenis* spiders, took 70 people 4 years to prepare from the silk collected from over 1 million spiders at a cost of over half a million US dollars [26]). Unfortunately attempts to farm spiders on an industrial scale have been fruitless owing to their cannibalistic nature, yet the determination of the primary structures of natural spider silk proteins has allowed the production of spider silk-like proteins using recombinant DNA technology on an industrial scale [27]. Recombinant DNA technology has also been used to prepare chimaeric/hybrid proteins incorporating silkworm silk-like or spider silk-like sequences and other sequences that enhance the proteins solubility, or improve biomineralization/cell adhesion of materials prepared from the proteins [28].

1.4. Processing silk proteins

Natural silkworm silk fibers require very little processing prior to using them in the textiles industry (e.g. dyeing [29], [30] and [31], or chemical modification to render them waterproof [32] and [33]). However, in order to prepare alternative material morphologies (e.g. films, foams, hydrogels, fibers with nanometer scale diameters, spheres or capsules) or composite materials, it is typically necessary to dissolve the proteins in a solvent capable of denaturing the protein (by breaking the strong intermolecular hydrogen bonds – stacks of β -sheets are particularly important for silk protein interaction), such as concentrated aqueous solutions of inorganic/organic salts, fluorinated solvents, ionic liquids or strong acids [3], [34] and [35]. Once the silk protein is dissolved it can be processed into a variety of different material morphologies; for example silk fibers with μm scale diameters can be prepared by hand-drawing or dry-/wet-spinning, and silk fibers with nm scale diameters can be prepared by electrospinning. Silk films can be prepared by casting and dip-/spin-coating; silk hydrogels can be prepared by exposure of aqueous solutions of silk proteins (after dialysis to remove any denaturant) to various stimuli including salt, shear, sonication or up-concentration; silk foams can be prepared by freeze-drying hydrogels, gas foaming or salt-leaching; silk spheres can be prepared by electrospraying or precipitation upon addition of a poor solvent to a solution of silk, and silk capsules can be prepared by adsorption of the protein at the interface of a water-in-oil emulsion. The materials formed by the above processes are often treated with alcohols (typically methanol) or aqueous solutions of kosmotropic salts (such as potassium phosphate) in order to induce β -sheet formation in the material, which reduces the solubility in water and changes the mechanical properties (Fig. 3) [3], [34] and [35]. We refer interested readers to a selection of recent reviews including sections on the processing of silk-based materials [3], [11], [34] and [35].

Start: protein source



Washed natural
silk fiber



Recombinantly
produced silk

Route 1) Processing natural silk fibers for textiles applications

- A) Chemical modification
- B) Dyeing

Route 2) Processing silk proteins into other materials morphologies

2i) Dissolve the protein in a suitable solvent:

- A) Aqueous denaturant solution (e.g. 9M lithium bromide) - optional dialysis
- B) Fluorinated solvent (e.g. hexafluoroisopropanol)
- C) Ionic liquid (e.g. 1-ethyl-3-methylimidazolium thiocyanate)
- D) Strong acid (e.g. formic acid)

2ii) Remove undissolved material from the solution

- A) Centrifugation
- B) Filtration

2iii) Add other component

- A) Add silk solution to other component in solid state
- B) Mix silk solution with other component in solution

2iv) Prepare material

- Capsules) adsorption of silk at the water-oil interface of water-in-oil emulsions
- Fibers) electrospinning, dry-/wet-spinning, or hand-drawing
- Films) casting, dip-/spin-coating
- Foams) freeze-drying, gas-foaming or salt-leaching
- Hydrogels) exposure to salt, shear, sonication or up-concentration
- Spheres) electrospraying or precipitation upon addition of a poor solvent to a solution



Capsule



Fiber



Film



Foam



Hydrogel



Sphere

2v) Optional post-treatment of material

- A) Methanol / potassium phosphate (induce β -sheet formation in silk)
- B) Heat (anneal polymer / induce β -sheet formation in silk)

Fig. 3.

Processing conditions of naturally/recombinantly produced silk proteins.

2. Man-made composite materials based on silk proteins

As noted in Section 1, the mechanical properties and biocompatibility of naturally occurring silkworm and spider silk protein-based fibers have allowed humans to use such fibers for millennia for a variety of applications. The development of new composite materials based on silk proteins has been driven in part by the necessity to address the shortcomings of natural silk fibers for certain applications (e.g. in textiles) and our ability to utilise the proteins as building blocks of materials with interesting new applications.

2.1. Man-made composites based on silk proteins and synthetic polymers

2.1.1. Man-made composites based on silk proteins and non-biodegradable synthetic polymers

Our ability to synthesize diverse structures with tunable properties has made synthetic polymer based materials absolutely ubiquitous in our daily lives. Indeed the first reports of silk-based composite fibers in 1956 utilized non-biodegradable synthetic polymers (poly(acrylonitrile) [36] in this case) to improve their properties for textiles applications, and since this time a variety of other materials morphologies have been prepared based upon silk proteins in combination with non-biodegradable synthetic polymers. Table 2 highlights a small selection of interesting examples.

Table 2.

Examples of man-made composite materials based on silk proteins and synthetic polymers.

Morphologies	Components	Improvement vs. silk	References
Fibers, films or spheres	Either A) <i>B. mori</i> fibroin or B) <i>N. clavipes</i> spidroin with carbon nanotubes	Electrical conductivity, mechanical properties	[37], [38], [39], [40], [41], [42], [43], [44], [45], [46], [47], [48] and [49]
Fibers	<i>B. mori</i> fibroin and poly(pyrrole)	Electrical conductivity	[69]
Films	<i>B. mori</i> fibroin and poly(vinyl alcohol)	Water permeability	[74]
Films	<i>B. mori</i> fibroin and pellethane heparin	Biocompatible coating with anticoagulant properties	[94]
Foams	<i>B. mori</i> fibroin and poly(aspartic acid)	Biomineralization (improved mech. properties) needed for tissue engineering	[78], [79] and [80]

Morphologies	Components	Improvement vs. silk	References
Foams	<i>B. mori</i> fibroin and poly(ϵ -caprolactone)	Cell adhesion and proliferation, i.e. tissue engineering	[85]

[Table options](#)

2.1.1.1. Carbon nanotubes

Carbon nanotubes are a recently discovered allotrope of carbon with nanometer scale diameters and lengths that can be many thousands of times longer. Carbon nanotubes are typically categorized as either single-walled nanotubes (SWNT) or multi-walled nanotubes (MWNT), both of which exhibit extraordinary strength and heat conduction [\[37\]](#) and [\[38\]](#).

Fibers composed of *B. mori* fibroin and SWNT were produced by electrospinning blends of 12 wt% *B. mori* fibroin and between 0.5 and 5 wt% SWNT in formic acid solution, resulting in smooth fibers with diameters of 20–250 nm. There was evidence for both random coil and β -sheet secondary structures in the fibroin which acted as a matrix for the dispersion of SWNT that were roughly aligned with the long axis of the fiber. It was found that by including 1 wt% of SWNT it was possible to improve the Young's modulus of the resultant fibers by up to 460% at the expense of the strength and strain to failure; all other ratios of fibroin and SWNT were observed to worsen the mechanical properties of the fibers due to poor alignment and dispersion of the SWNT within the fibroin matrix [\[39\]](#). Likewise fibers composed of *B. mori* fibroin and MWNT can be produced by electrospinning from formic acid solution, resulting in fibers with improved Young's modulus and tensile strength at the expense of the elongation at break [\[40\]](#).

Films composed of *B. mori* fibroin and MWNT were cast from aqueous solution, and there was evidence for random coil, α -helix and β -sheet secondary structures in the fibroin. There were notable improvements in tensile modulus and tensile strength at the expense of elongation at break [\[41\]](#). Films composed of recombinantly produced proteins based on the major ampullate spidroins of *N. clavipes* with SWNT functionalized with octadecylamine were cast from hexafluoroisopropanol solution and subsequently vacuum dried. The as-cast films had very low β -sheet content (as hexafluoroisopropanol induces α -helix formation in proteins [\[42\]](#)) and consequently had poor mechanical properties (stiffness, strength and ductility), however, including small quantities (volume fraction of ca. 0.1%) of SWNT improved these properties by 14–45% [\[43\]](#). Fibers of *B. mori* fibroin with a MWNT coating were used to reinforce films cast from polymer melts of poly(butylene succinate). It was shown that a small amount of silk fibers (ca. 3 wt%) dramatically improved the tensile strength and modulus of the composites [\[44\]](#).

Fibers and microspheres of *B. mori* fibroin have been coated with MWNTs by immersion of the fibers or microspheres in aqueous suspensions of the MWNTs, followed by washing and drying. Such coatings were stable to both extensive washing and sonication due to the formation of hydrogen bonds between the fibroin and the carboxylic acids on the surface of the nanotubes (introduced during the nanotube purification procedure) [\[40\]](#), [\[45\]](#) and [\[46\]](#).

Hydrogels composed of *B. mori* fibroin and MWNT can be prepared by sonication of the nanotubes in 5 wt% solutions of *B. mori* fibroin at pH 12 followed by reduction of the pH of the solution to pH 4.5. Interestingly this gelation process could be reversed by increase of the pH of the solution to pH 12, although the authors do not mention if the fibroin is hydrolyzed

under such conditions [47] and [48]. Freeze-drying such hydrogels yielded foams in which aggregated MWNT were embedded in a continuous fibroin matrix. The MWNT interacted with the fibroin via hydrogen bonding interactions between the fibroin and the carboxylic acids on the surface of the nanotubes [49].

2.1.1.2. Nylon

Nylon 66 is a cheap and strong polyamide that is widely used in the plastics and textiles industries. Films composed of blends of *B. mori* fibroin and nylon 66 were cast from formic acid solution and vacuum dried. The polymers in the resulting films were crystalline and appeared to be homogeneously distributed at all blend ratios other than 50:50 fibroin:nylon [50]. It was also possible to prepare particles composed of *B. mori* fibroin and nylon 66 by precipitation from formic acid solutions of the polymers upon the addition of acetone, followed by filtration, washing with acetone and water drying; yielding particles in which the polymers were phase separated [50].

2.1.1.3. Poly(acrylamide)

Poly(acrylamide) is a highly water absorbent polymer used in both bulk applications such as waste water treatment and if highly purified (to remove the neurotoxic monomer acrylamide) as soft contact lenses. Films composed of *B. mori* fibroin and poly(acrylamide) were cast from aqueous solution, incubated in a humid atmosphere to induce β -sheet formation in the fibroin, and finally vacuum dried. The polymers were phase separated, with spherical poly(acrylamide) particles dispersed in a continuous phase of fibroin. Films with a poly(acrylamide) content of up to 25 wt% showed improved mechanical stability compared to films of fibroin alone [51].

2.1.1.4. Poly(acrylonitrile)

Fibers composed of poly(acrylonitrile) and its copolymers are cheap, antibacterial and stable to photo-oxidation, and are commonly used for textiles and the reinforcement of composite materials; however, these polymers absorb little moisture (due to their hydrophobicity) and collect static electricity. Fibers composed of *B. mori* fibroin and poly(acrylonitrile) were produced by wet-spinning solutions of 8.5 wt% fibroin/poly(acrylonitrile) in aqueous ZnCl_2 (56 wt%) into an aqueous coagulation bath [36]. The fibers were air-dried for a few hours, drawn to ca. six times their original length, washed first with aqueous Na_2CO_3 (10 wt%) (neutralizing the ZnCl_2), then with water and finally air-dried. The resultant fibers were transparent with circular cross-sections and were smooth (except at fibroin:poly(acrylonitrile) ratios of 20:80). X-ray diffraction studies showed that the polymers were neither highly crystalline nor strongly oriented, and scanning electron microscopy (SEM) studies carried out later indicated the polymers to be phase separated [52]. Electrical resistivity measurements indicated that the fibers were contaminated with residual Zn; this problem could be overcome using sodium thiocyanate as the protein denaturant in the spinning dope. Subsequent studies showed that the fibers had a core-sheath structure, with a fibroin-rich sheath, and a poly(acrylonitrile) rich core containing long fibrils of fibroin aligned with the direction of the fiber (caused by elongational flow forces during the fiber spinning process). The resulting fibers had mechanical properties usable in the textiles industry.

Compatibilization of *B. mori* fibroin and poly(acrylonitrile) can be achieved through the addition of small amounts (ca. 2 wt%) of fibroin-graft-poly(acrylonitrile) copolymers to

mixtures of fibroin and poly(acrylonitrile). The best compatibilization was achieved with a fibroin-graft-poly(acrylonitrile) copolymer bearing (on average) one long poly(acrylonitrile) chain (of ca. 106 kDa), which almost eliminates phase separation. When preparing fibers based on mixtures of *B. mori* fibroin and poly(acrylonitrile) for textiles applications, complete compatibilization is undesirable as it reduces the handle and luster of the fibers imparted by the silk sheath. A copolymer bearing (on average) two short poly(acrylonitrile) chains (of ca. 65 kDa) was used to successfully improve the tensile strength by 50% (relative to the fiber in the absence of the compatibilizer) whilst maintaining a fibroin-rich sheath [53] and [54].

Particles composed of *B. mori* fibroin and poly(acrylonitrile) were prepared by precipitation from aqueous solutions of the polymers upon the addition of methanol (inducing β -sheet assembly in the fibroin), followed by filtration, dialysis (to remove methanol) and vacuum drying, yielding particles in which the polymers were phase separated. In contrast, the polymers in particles composed of *B. mori* fibroin and poly(acrylonitrile-co-methyl acrylate) were more homogeneously distributed due to the existence of hydrogen bonds between the polymers [55].

2.1.1.5. Poly(allylamine)

Poly(allylamine) is a polycationic polymer that is highly soluble in water and utilized widely in biomedical applications. Films composed of *B. mori* fibroin and poly(allylamine) were cast from aqueous solution and found to be more stable in water than films composed only of poly(allylamine). The polymers were compatible, yet Fourier transform infrared (FTIR) spectroscopic studies indicated only weak interactions between the polymers [56].

2.1.1.6. Poly(epoxides)

Poly(epoxides) are commonly used as adhesives, coatings and structural materials. Incorporation of waste silk fabric (1 wt%) into films cast of Araldite HY960 epoxy resin (with a monomer:hardener ratio of 5:1) yielded resins with improved stiffness at the expense of tensile strength, ductility and elongation at break [44]. In contrast, the incorporation of waste silk fabric (degummed *B. mori* fibroin fibers) (up to 25 wt%) into films cast from polymer melts of an epoxy resin (synthesized from Vantico's LY556 monomer and HY951 hardener) led to increases in tensile strength, tensile modulus and elongation at break. The toughness could be further increased by addition of either poly(methyl methacrylate) (4 wt%) or poly(carbonate) (6 wt%) [57].

2.1.1.7. Poly(ethylene oxide)

Poly(ethylene oxide) (also known as poly(ethylene glycol)) polymers are cheap, water-soluble and biocompatible, with a wide variety of applications. Fibers composed of *B. mori* fibroin and poly(ethylene oxide) (900 kDa) were produced by electrospinning aqueous solutions of 2–8 wt% *B. mori* fibroin with up to 1.8 wt% poly(ethylene oxide), yielding smooth fibers with diameters of 590–910 nm. The polymers in the as-electrospun fibers were phase separated, with island-like regions of crystalline poly(ethylene oxide) (elongated along the long axis of the fiber) and an unstructured silk fibroin matrix (evaporation of the solvent during electrospinning is fast, and silk fibroin crystallizes slowly in water relative to poly(ethylene oxide)). Treatment of these fibers with 90/10 (v/v) methanol/water rendered the surface of the fibers rough, due to partial dissolution of the poly(ethylene oxide) and

simultaneous induction of β -sheet formation in the fibroin [58] and [59]. Almost all of the poly(ethylene oxide) could be removed by subsequently washing the fibers with water.

It is also possible to electrospin fibers with a core-sheath morphology by a two-fluid electrospinning technique (one fluid containing *B. mori* fibroin and the other poly(ethylene oxide)) using water as the common solvent. The two-fluid electrospinning process is the key to producing a core filament of unblended, unstructured fibroin and allowed a variety of core-sheath diameters to be prepared [60]. Exposure of the as-spun fibers to a humid atmosphere plasticized the fibers and allowed β -sheet formation in the fibroin, and the poly(ethylene oxide) sheath could be removed by subsequently washing the fibers with water.

Films composed of *B. mori* fibroin and poly(ethylene oxide) were cast from aqueous solution, treatment with methanol induced β -sheet formation in the fibroin. At a blend ratio of 98:2 fibroin:poly(ethylene oxide) a homogenous film was formed with improved tensile strength and elongation at break in comparison with films composed solely of fibroin. In films with a higher poly(ethylene oxide) content the polymers were phase separated, with fibroin particles homogeneously dispersed in a poly(ethylene oxide) matrix. Films with a poly(ethylene oxide) content of greater than 30 wt% had improved elongation at break at the expense of tensile modulus and tensile strength. As previously demonstrated with the fibers, the poly(ethylene oxide) matrix could be washed away in water, yielding a porous film composed of fibroin [61] and [62].

Hydrogels composed of *B. mori* fibroin and poly(ethylene oxide) were prepared simply by mixing aqueous solutions of the polymers. Hydrogelation occurred faster as the poly(ethylene oxide) content increased due to restriction of the mobility of the fibroin, encouraging the formation of β -sheets between the proteins [63]. Cross-linking of the poly(ethylene oxide) polymers was demonstrated to significantly improve the mechanical properties (tensile strength and elongation at break) and water swellability of the hydrogels [64], [65] and [66].

Hydrogels composed of *B. mori* fibroin and a poly(ethylene oxide)-poly(propylene oxide) copolymer (Pluronic F-127) were prepared simply by mixing acidified (pH 4) aqueous solutions of the polymers. The viscosity of the hydrogels was found to increase as the concentration of Pluronic F-127 increased [67].

2.1.1.8. Poly(pyrrole)

Poly(pyrrole) is a biocompatible conducting polymer discovered in the 1960s currently investigated for a wide variety of biomedical applications [68]. Forcibly silked major ampullate silk fibers (produced by *Nephila edulis* spiders) were dip-coated with poly(pyrrole) followed by air drying, with almost no detrimental effect upon the mechanical properties of the fibers [69]. Such composite fibers may find application in electrically stimulated tissue scaffolds or drug delivery devices [68].

2.1.1.9. Poly(styrene)

Poly(styrene) is cheap and utilized in a wide variety of applications. Films composed of *B. mori* fibroin and ordered arrays of poly(styrene) microspheres were prepared by casting aqueous solutions of fibroin onto the microspheres, followed by air drying and subsequent treatment with ethanol to induce β -sheet assembly in the fibroin. Fibroin foams with three-dimensionally ordered pores could be produced by washing these films with toluene which

selectively dissolved the poly(styrene) microspheres, yielding foams that were superhydrophobic and mechanically stable [70].

2.1.1.10. Poly(vinyl alcohol)

Poly(vinyl alcohol) is a cheap, water-soluble, non-toxic, biocompatible and degradable polymer. Fibers composed of blends of *B. mori* fibroin and poly(vinyl alcohol) were produced by wet-spinning solutions of 19 wt% fibroin/poly(vinyl alcohol) in formic acid into a methanol coagulation bath where they were left overnight to allow solidification of the fiber and β -sheet formation in the fibroin [71]. Once solid, the fibers were plasticized in hot water (70 °C), drawn to ca. 4.5 times their original length, and finally air-dried under tension. The resultant white fibers had circular cross-sections (with diameters of between 220 and 270 μm) and were smooth. X-ray diffraction studies indicated that the fibroin was crystalline and that the poly(vinyl alcohol) was amorphous, and SEM studies demonstrated that the polymers were not macroscopically phase separated. Wet-spun fibers composed solely of *B. mori* fibroin had reasonable breaking strength, but were brittle and inflexible. Blending up to 50 wt% of poly(vinyl alcohol) was shown to increase the tenacity and elongation at break of the fibers (at the expense of the breaking strength).

It is possible to prepare films by casting aqueous solutions of *B. mori* fibroin and poly(vinyl alcohol). The films were annealed at 200 °C which crystallized the poly(vinyl alcohol) without affecting the conformation of fibroin (as shown by FTIR), followed by immersion in methanol to induce β -sheet formation in the fibroin (also shown by FTIR). The polymers in the films were phase separated, and the mechanical properties were consequently non-additive [72] and [73]. It is also possible to prepare transparent films by casting mixtures of *B. mori* fibroin and poly(vinyl alcohol) in hexafluoroisopropanol solution. At a weight ratio of 80:20 fibroin:poly(vinyl alcohol) the film was homogenous, with a higher β -sheet content than films cast from fibroin alone. At higher weight ratios the polymers were phase separated, with fibroin particles (of 1–7 μm) dispersed in a continuous phase of poly(vinyl alcohol). An increase in the poly(vinyl alcohol) content increases the hydrophilicity of the films (a consequence of which is that they swell more in water), the degree of swelling could be reduced by annealing the films at 70–80 °C which induces β -sheet formation in the fibroin (as shown by FTIR) due to the slow removal of residual water from the film (as shown by differential scanning calorimetry (DSC)), thereby increasing their water resistance [74].

Hydrogels composed of *B. mori* fibroin and poly(vinyl alcohol) were prepared by mixing aqueous solutions of the polymers, incubation at –50 °C for 2 h, freeze-drying and re-hydration. The elongation at break of the hydrogels was demonstrated to increase as the poly(vinyl alcohol) content increased, yet the strength was unaffected [75]. Foams composed of *B. mori* fibroin and poly(vinyl alcohol) have been prepared by either air or freeze-drying the aforementioned hydrogels. Deeper freezing increased the β -sheet content of the foams (as did air drying) and consequently the mechanical properties (strength and elongation at break) were improved relative to the freeze-dried foams. Moreover, the strength and elongation at break increased as the poly(vinyl alcohol) content increased [75] and [76].

2.1.2. Man-made composites based on silk proteins and biodegradable synthetic polymers

Biodegradable synthetic polymers are more attractive than their non-biodegradable brethren for many materials applications, particularly when considering their disposal at the end of

their useful lifetimes as they pose fewer environmental hazards. In this context a number of researchers have investigated the properties of composite materials composed of silk proteins and biodegradable polymers for a variety of applications [77]. [Table 2](#) contains a small selection of interesting examples.

2.1.2.1. Poly(aspartic acid)

The poly(aspartic acid) domains of certain natural proteins are known to be involved in biomineralization processes. Foams composed of *B. mori* fibroin and poly(aspartic acid) were prepared by casting mixtures of the polymers in aqueous solution into a container packed with sodium chloride, incubation for 24 h at room temperature (allowing the formation of β -sheets between the fibroin), followed by salt-leaching by washing with water. The foams were β -sheet rich, and had a continuous network of pores of approximately 750 μm [78], [79] and [80]. Inclusion of poly(aspartic acid) in such composite materials is beneficial for their subsequent biomineralization, as discussed later in this review.

2.1.2.2. Poly(ϵ -caprolactone)

Poly(ϵ -caprolactone) is a biodegradable polyester approved by the US Food and Drug Administration (FDA). Films composed of poly(ϵ -caprolactone) reinforced with naturally spun *B. mori* fibroin fibers were prepared by melt-mixing of the poly(ϵ -caprolactone) and fibroin fibers followed by hot pressing (at 140 °C) into the desired form. The films with 35–45 wt% fiber content had optimal mechanical properties [81], [82] and [83]. Subsequent studies on such systems showed that exposure to electron beams generates radicals on both the poly(ϵ -caprolactone) and fibroin leading to both cross-linking and degradation of the polymers, which resulted in moderate increases in tensile strength and flexural strength below 150 kGy, and notable decreases above 150 kGy [84]. Composite materials in which a *B. mori* fibroin foam filled the pores of a poly(ϵ -caprolactone) foam were prepared by freeze-drying poly(ϵ -caprolactone) foams filled with aqueous solutions of fibroin [85].

2.1.2.3. Poly(ϵ -caprolactone-co-d,l-lactide)

Poly(ϵ -caprolactone-co-d,l-lactide) is a biodegradable polymer of great popularity that degrades relatively quickly. Films composed of *B. mori* fibroin and poly(ϵ -caprolactone-co-d,l-lactide) were prepared by casting suspensions of *B. mori* fibroin particles in solutions of poly(ϵ -caprolactone-co-d,l-lactide) in acetone, followed by vacuum drying. The β -sheet rich fibroin particles were homogeneously distributed within the poly(ϵ -caprolactone-co-d,l-lactide) matrix, and the films became more opaque as the fibroin content increased. Furthermore, the mechanical properties (storage modulus and hardness) of the films were observed to improve as the fibroin content increased, indicative of hydrogen bonding between the fibroin particles dispersed in the poly(ϵ -caprolactone-co-d,l-lactide) matrix [86].

2.1.2.4. Poly(carbonate-urethane)

Poly(urethane) elastomers have been used extensively in biomedical applications due to their biocompatibility and mechanical properties. Poly(ester urethanes) are unsuitable for long-term implantation as they are hydrolyzed relatively quickly *in vivo*; poly(ether urethanes) are more stable to hydrolysis, but observed to oxidize *in vivo* after extended periods; poly(carbonate urethanes) by contrast are more stable to both hydrolysis and oxidation *in vivo* and have more potential for use as long-term implants. Bilayer films composed of *B. mori* fibroin and medical grade poly(carbonate-urethane), were prepared by: first casting films of

poly(carbonate-urethane) from solution in tetrahydrofuran/dioxane, drying, and applying a second layer of fibroin (cast from aqueous solution), drying and finally treating with methanol (inducing β -sheet assembly in the fibroin). The fibroin surface was slightly rough and stable to prolonged incubation in phosphate buffered saline solutions [87], [88] and [89].

2.1.2.5. Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid) copolyesters have been developed commercially for a wide range of biomedical applications. Poly(lactic-co-glycolic acid) microspheres (with diameters of ca. 53 μm) were coated with silk by adsorption (due to hydrophobic interactions) from an aqueous solution of *B. mori* fibroin (0.1% w/v), prior to repetitive centrifugation/washing steps, exposure to aqueous methanol (inducing β -sheet assembly in the fibroin), and drying under a stream of nitrogen (two further coats of fibroin were applied using the same procedure). The silk coating was approximately 1 μm thick and stable to prolonged incubation in phosphate buffered saline solution [90].

2.1.2.6. Poly(lactic acid)

Poly(lactic acid) which is also known as poly(lactide), has been developed for a variety of biomedical applications; homopolymers of l-lactide or d-lactide tend to be crystalline and degrade slowly, whereas copolymers tend to be amorphous and degrade faster. The good mechanical properties of the homopolymers make them ideal for load bearing applications, whereas the copolymers are preferred for drug delivery applications.

Films composed of *B. mori* fibroin and poly(lactic acid) were cast from aqueous dioxane solution and dried at 30 °C and 50% relative humidity for 48 h. The polymers were phase separated with poly(lactic acid) particles dispersed in a continuous phase of fibroin, and they were shown to interact via hydrogen bonding interactions. Furthermore, mixing up to 10 wt% of poly(lactic acid) was demonstrated to moderately improve the tensile strength and elongation at break of the films and decrease the hydrophilicity of the films [91].

Foams composed of *B. mori* fibroin and poly(l-lactide) were prepared by freeze-drying dioxane solutions of blends of soluble poly(l-lactide) and insoluble β -sheet rich fibroin particles (5–50 μm). The fibroin particles were homogeneously distributed throughout the poly(l-lactide) foam and were shown to interact with the poly(l-lactide) matrix via hydrogen bonding interactions; furthermore, the presence of fibroin increased the hydrophilicity of the foams [92].

Fibers composed of *Agelena labyrinthica* spidroin and poly(d,l-lactide) were produced by electrospinning emulsions of (*A. labyrinthica* spidroin in formic acid)-in-(poly(d,l-lactide) in acetone), followed by drying under vacuum for 4 days, yielding fibers with diameters of 89–1050 nm. The polymers in the as-electrospun fibers were phase separated, and the proteins adopted predominantly α -helical/random coil conformations (with some β -sheet content). At low weight ratios (of <15 wt% spidroin:poly(d,l-lactide)) this resulted in granular regions of silk spidroin within a poly(d,l-lactide) matrix, whereas at higher weight ratios (of >15 wt% spidroin:poly(d,l-lactide)) the fibers had a core-sheath morphology (in which the core was spidroin). As the spidroin content increased, so did the mechanical properties (tensile strength and elongation at break) of the resultant fibers [93].

2.1.2.7. Poly(urethane) – pellethane

Pellethane is the trade name of a family of polyurethanes that are biodegradable, thermoplastic elastomers. Films composed of pellethane (2363-80A), *B. mori* fibroin particles (of ca. 3.6 μm) and the sodium salt of heparin (10 wt%) were cast from DMF solution, and the solvent removed at room temperature, followed by a final drying step at 80 °C for 24 h, resulting in films that were relatively smooth in which the fibroin and heparin were homogenously dispersed in a matrix of pellethane [94].

2.2. Man-made composites based on silk proteins and biopolymers

2.2.1. Man-made composites based on silk proteins and other proteins

As described in Section 1, naturally occurring silk fibers are composite materials based upon the combination of a variety of proteinaceous components (fibroins and sericins in the case of silkworm cocoon fibers, or major ampullate spidroins 1/2 (or analogues), minor ampullate silk-like proteins, glycoproteins and lipids in the case of spider's dragline fibers). *In vitro* a variety of other composite materials have been prepared based upon mixtures of silk proteins and other proteins/enzymes that have a number of exciting applications. Table 3 shows some interesting examples.

Table 3.

Examples of man-made composite materials based on silk proteins and biopolymers.

Morphologies	Components	Improvement vs. silk	References
Fibers or spheres	<i>B. mori</i> fibroin and either bone morphogenic protein-2 or insulin-like growth factor	Tissue engineering: growth factor determined cell-differentiation	[118] and [119]
Films	<i>B. mori</i> fibroin and either A) glucose oxidase or B) horseradish peroxidase or C) β -galactosidase	Catalytic activity of the respective enzyme	[104], [105], [106], [107], [108], [161] and [218]
Films	<i>B. mori</i> fibroin and green fluorescent protein	Non-linear optical properties	[117]
Microspheres embedded in foams or hydrogels	Either A) <i>A. mylitta</i> fibroin or B) <i>B. mori</i> fibroin and alginate	Drug delivery without burst profile	[119] and [130]
Fibers	<i>B. mori</i> fibroin cellulose	Cheap textiles from waste materials	[131] and [137]

[Table options](#)

2.2.1.1. Collagen

Collagen is the most abundant protein in mammals, and is particularly important in the extracellular matrix. Fibers composed of *B. mori* fibroin and collagen were produced by electrospinning solutions of *B. mori* fibroin and collagen in hexafluoroisopropanol. Incubation in an atmosphere of water and glutaraldehyde vapor, followed by immersion in an aqueous solution of glycine (to block unreacted aldehyde groups), induced β -sheet formation in the fibroin and chemically cross-linked the proteins. The samples were finally vacuum dried, resulting in smooth fibers with average diameters of 320–360 nm [95] and [96].

Films composed of *B. mori* fibroin and recombinant human-like collagen were prepared by casting from warm (50–60 °C) aqueous solutions, followed by drying at 70 °C. The films cast from fibroin alone contained some β -sheet crystals, probably due to the high temperature drying/annealing process. The presence of collagen in the films resulted in the formation of hydrogen bonds between the two proteins (at the expense of β -sheet content), and the films were consequently slightly less mechanically stable than films cast from fibroin alone. Recombinant human-like collagen is more hydrophilic than fibroin, so the surfaces of the films were more hydrophilic than those cast from fibroin alone [97]. Films composed of *B. mori* fibroin and bovine collagen cast under analogous conditions showed moderate improvements in the wet-state flexibility of the films at the expense of strength and stiffness [98]. Films composed of *B. mori* fibroin and rat-tail collagen were prepared by casting from aqueous acetic acid solutions, air drying, treating with methanol to induce β -sheet formation in the fibroin, and air drying again. The films were optically transparent, smooth and hydrophilic (water uptake of ca. 30% dry weight) [99].

Foams composed of *B. mori* fibroin and bovine collagen were prepared by freeze-drying aqueous solutions of the polymers. The foams were subsequently treated with methanol (inducing β -sheet assembly in the fibroin) and air-dried. The polymers were homogeneously distributed in the foams and interacted via hydrogen bonds. The water swellability of the foams increased as the collagen content increased. The mechanical properties (yield stress and compressive moduli) increased with increasing collagen content [100], [101] and [102]. Subsequent studies with recombinantly produced human-like collagen yielded similar results [103].

2.2.1.2. Enzymes

Enzymes are biomolecules that catalyze certain chemical reactions, and their incredible catalytic efficiency has resulted in numerous biomedical and technical applications of enzymes that are easy to isolate. Films composed of *B. mori* fibroin and the enzymes Glucose oxidase or Horseradish peroxidase were prepared by casting from aqueous solutions, followed by exposure to aqueous methanol (inducing β -sheet assembly in the fibroin), and air drying (Table 3) [104], [105], [106], [107] and [108]. The enzymes were homogeneously distributed and remained trapped within the β -sheet cross-linked fibroin matrix even after long periods of exposure to water.

2.2.1.3. Fibroins of different species

As outlined in Section 1, mankind has harvested *B. mori* silkworm fibroin for thousands of years for use in diverse applications. Other species of silkworms produce different fibroins for protective cocoons that have in recent years been investigated with a view to understanding the molecular basis for the different mechanical properties of their silks, and their potential use in various medical applications.

Films composed of *B. mori* fibroin were prepared by casting from aqueous solutions (of soluble fibroin and insoluble β -sheet rich fibroin nanoparticles) followed by drying at 45 °C. It was demonstrated that the presence of β -sheet rich fibroin nanoparticles induced β -sheet formation in the films, yielding smooth films that were insoluble in water [109].

B. mori and *A. pernyi* fibroins have notably different primary structures. The glycine content being higher in *B. mori* fibroin, and the content of acidic and basic amino acids being higher in *A. pernyi* fibroin; moreover, the β -sheet forming segments of *B. mori* fibroin are composed mainly of (Gly-Ala)_n repeats, whereas in *A. pernyi* fibroin they are composed mainly of (Ala)_n repeats. Films composed of *B. mori* and *A. pernyi* fibroins were prepared by casting from aqueous solutions (of native proteins extracted from the lumen of the silkworms), followed by incubation in a humid atmosphere (allowing the formation of β -sheets in the fibroins) and vacuum drying. Various techniques demonstrated that the fibroins were phase separated in the films [110].

Films composed of *B. mori* fibroin and an engineered hybrid protein-based on the repeat sequence of *N. clavipes* spidroin and an integrin-binding Arg-Gly-Asp motif (more commonly referred to as an RGD motif), were prepared by spin-coating from hexafluoroisopropanol solutions, and either left to dry at room temperature or annealed by incubation in a hot (70 °C) humid atmosphere. The proteins in unannealed films were predominantly randomly coiled, whereas those in the annealed films were β -sheet rich with β -sheet content increasing with spidroin content [111].

2.2.1.4. Gelatin

Gelatin is produced by hydrolysis of collagen, and used as a gelling agent in drug formulations, food, and photography. It was possible to induce hydrogel formation in aqueous solutions containing *B. mori* fibroin and gelatin by exposure to methanol (inducing β -sheet formation in the fibroin) [112] and [113]. It is also possible to prepare tubes of gelatin-based hydrogel reinforced with naturally spun *B. mori* fibroin fibers, yielding tubes with mechanical properties similar to those of arteries [114]. Films composed of *B. mori* fibroin and gelatin were cast from aqueous solutions, followed by treatment with aqueous methanol and vacuum drying. The proteins were homogeneously distributed and were demonstrated to interact via hydrogen bonds, and blend films were more thermally and mechanically stable than the films cast from either protein alone [115]. Subsequent studies demonstrated it is possible to prepare microporous fibroin films by dissolution of the gelatin in aqueous phosphate buffered saline solution at 37 °C [116].

2.2.1.5. Green fluorescent protein

Green fluorescent protein fluoresces green when exposed to blue light, and is used extensively in cell and molecular biology. Films composed of *B. mori* fibroin and green fluorescent protein were cast from aqueous solution and air-dried (Table 3). The films had rough surfaces and the green fluorescent protein was inhomogeneously distributed throughout the fibroin matrix [117]. Such composite materials have potentially interesting nonlinear optical applications.

2.2.1.6. Growth factors

Bone morphogenic proteins are a group of growth factors and cytokines that induce the formation of bone and cartilage; such proteins can be produced recombinantly and are currently under development for medical and dental applications. Fibers composed of *B. mori* fibroin, poly(ethylene oxide) and bone morphogenic protein-2 were electrospun from aqueous solution, immersed in methanol (rendering the fibers insoluble in water due to β -sheet assembly in the fibroin) then dried, yielding smooth fibers with diameters of approximately 570 nm ([Table 3](#)). The polymers in the fibers were phase separated with the proteins in one phase and the poly(ethylene oxide) in another [\[118\]](#).

More recently, bone morphogenic protein-2 and recombinant human insulin-like growth factor-1 were incorporated in *B. mori* fibroin microspheres which were subsequently incorporated within either an alginate gel or a *B. mori* fibroin foam [\[119\]](#).

2.2.1.7. Keratin

Keratins are tough structural proteins produced by animals. Fibers composed of *B. mori* fibroin and oxidized merino wool keratin were produced by electrospinning solutions of *B. mori* fibroin and keratin in formic acid. The as-spun fibers were smooth with diameters between 160 and 900 nm, and the proteins adopted predominantly α -helical/random coil conformations [\[120\]](#). Immersion of the fibers in methanol induced β -sheet formation in the fibroin, and incubation in an atmosphere of formaldehyde vapor chemically cross-linked the proteins, further improving their mechanical stability [\[121\]](#).

Films composed of *B. mori* fibroin and merino wool keratin were prepared by casting from formic acid solution. The presence of the keratin promoted β -sheet formation in the fibroin (due to hydrogen bonding interactions between the proteins), as did treatment of the films with methanol [\[120\]](#), [\[122\]](#) and [\[123\]](#).

2.2.1.8. Sericin

As described in Section [1](#), sericins are glycoproteins produced by silkworms that are used as a protective glue coating on fibroin fibers [\[16\]](#). Films composed of *B. mori* fibroin and sericin were prepared by casting from aqueous solutions, followed by drying at 80 °C. The proteins in the as-cast films were amorphous and phase separated. Exposure of the films to methanol induced β -sheet formation in the fibroin, but this was retarded (although not prevented) by the presence of sericin in the films, suggesting the interaction of the polymers via hydrogen bonding interactions at the interface of the phase boundary [\[124\]](#).

2.2.1.9. Spidroins of different species

As described in Section [1](#), spidroins are fibrous proteins produced by spiders for a number of task-specific applications. Natural major ampullate silk fibers are composed of multiple proteins (see [Table 1](#)) [\[6\]](#) and [\[7\]](#), and in an effort to mimic the natural spinning process [\[125\]](#) we have used a microfluidic device (as its dimensions are similar to a spiders spinning duct) to prepare fibers composed of recombinantly produced spidroins (eADF-3 and eADF-4 [\[24\]](#)) that are based upon the consensus sequences of the major ampullate silks ADF-3 and ADF-4 of *A. diadematus* spiders. Using biomimetic conditions (combining increasing the phosphate concentration, pH decrease and elongational flow) we successfully prepared fibers with μ m scale diameters in which the proteins were β -sheet-rich [\[126\]](#).

We have also cast films from mixtures of such recombinantly produced spidroins in hexafluoroisopropanol solution. The as-cast films were α -helix rich and water-soluble, whereas those subsequently treated with either methanol or potassium phosphate were β -sheet rich and consequently insoluble in water [127].

2.2.2. Man-made composites based on silk proteins and polysaccharides

Polysaccharides are ubiquitous in nature and are typically utilized for energy storage or as structural elements in plant and cell walls that are often used in composite materials owing to their high natural abundance and relatively low cost. Table 3 shows some interesting examples.

2.2.2.1. Alginate

Alginate is a linear polysaccharide found in the cell walls of algae, and is utilized in the biomedical, food and textiles industries. Films composed of *B. mori* fibroin and sodium alginate were prepared by casting from aqueous solution, followed by incubation in a humid atmosphere (plasticizing the protein, allowing the formation of β -sheets in the fibroin). The mechanical properties of the films were optimal when the alginate content was 30 wt%, and the water absorbance of the films was notably improved by mixing alginate with fibroin [128]. Foams prepared by freeze-drying aqueous solutions of *B. mori* fibroin and sodium alginate had compressive moduli that were better than the foams composed of fibroin alone [129].

Alginate microspheres (with diameters of ca. 700 μm) were prepared by precipitating alginate in aqueous calcium chloride solution, and purified by shaking, filtration and washing in water. The resulting microspheres were coated with silk by adsorption (due to hydrophobic interactions) from an aqueous solution of *B. mori* fibroin (0.1% w/v), prior to repetitive centrifugation/washing steps, exposure to aqueous methanol (inducing β -sheet assembly in the fibroin), and drying under a stream of nitrogen (two further coats of fibroin were applied using the same procedure). The silk coating was approximately 10 μm thick and slightly rough [90]. It is also possible to prepare microspheres composed of alginate and *A. mylitta* fibroin by an analogous method (Table 3) [130], and more complex composite materials composed of such microspheres embedded in *A. mylitta* fibroin foams [130].

2.2.2.2. Cellulose

Cellulose is the most common organic compound on Earth, which is found in the cell walls of many plants, and is widely used in the textile industry (in the form of cotton, hemp or jute) as it is cheap, biodegradable and moisture absorbent. Fibers composed of *B. mori* fibroin and cellulose were produced by wet-spinning solutions of fibroin/cellulose in *N,N*-dimethylacetamide (containing 7 wt% LiCl) into an ethanol coagulation bath, and were washed for 3–4 days to assure extraction of all of the LiCl (Table 3) [131]. The resultant fibers had circular cross-sections (with diameters of between 20 and 36 μm) and were smooth. SEM studies demonstrated that the polymers were not macroscopically phase separated, and X-ray diffraction studies indicated that the crystalline fibroin was dispersed in an amorphous matrix of cellulose.

Films composed of *B. mori* fibroin and cellulose were prepared by casting aqueous cuprammonium hydroxide (Cuoxam) solutions of the polymers, followed by treatment with

acetone/acetic acid, and washes with aqueous glycerine and water, before air drying. Infrared absorption spectra showed intermolecular hydrogen bonds between the fibroin and cellulose, and peaks characteristic of the crystallinity of the polymers (β -sheet rich fibroin and cellulose II). The mechanical properties (tensile strength and elongation at break) were distinctly improved relative to the films cast from fibroin alone, despite minor degradation of the fibroin in the Cuoxam solution [132]. Subsequent studies demonstrated the potential to form porous films (composed of cellulose) via casting Cuoxam solutions of the polymers, followed by sequential treatment with: aqueous sodium hydroxide (removing the fibroin), aqueous sulfuric acid, acetone/acetic acid, aqueous glycerine, and water, before finally air drying [133] and [134].

It is also possible to prepare films composed of *B. mori* fibroin and cellulose by casting from N-methylmorpholine oxide solution, followed by treatment with aqueous alcohol, washing with water and air drying. DSC demonstrated the formation of intermolecular hydrogen bonds between the fibroin and cellulose, and that the fibroin was β -sheet rich [135].

2.2.2.3. Cellulose xanthate (viscose)

Cellulose xanthate (viscose) is a polymer popular in the textiles industry. Fibers composed of *B. mori* fibroin and viscose were produced by wet-spinning solutions of fibroin/viscose in aqueous sodium hydroxide solution into an acidic coagulation bath (aqueous sulfuric acid and ammonium sulfate) where they were left overnight to allow complete solidification of the fiber. The fibers were subsequently washed in various methanolic solutions before being air-dried under tension. The resultant white fibers had circular cross-sections, and the surface of the fibers had very fine grooves aligned with the fiber axis. The polymers were phase separated, with silk dispersed in a viscose matrix [136], and the fibers had mechanical properties suitable for certain textiles applications.

2.2.2.4. Chitin

Sodium *N*-acetylchitosan (chitin) is the main component of the cell walls of fungi, and the exoskeletons of arthropods and insects, that is utilized in adhesives, foods and various biomedical/filtration technologies. Fibers composed of *B. mori* fibroin and chitin were produced by wet-spinning solutions of fibroin/chitin in aqueous sodium hydroxide solution into an acidic coagulation bath (aqueous sulfuric acid and ammonium sulfate) where they were left overnight to allow complete solidification of the fiber. The fibers were subsequently washed in various methanolic solutions before being air-dried. The resultant white fibers had circular cross-sections, and the surface of the fibers was slightly rough. The mechanical properties of the fibers were poorer than those of either silk or chitin alone, but potentially usable in the textiles industry [137]. It is also possible to prepare fibers composed of *B. mori* fibroin, viscose and chitin in an analogous way [136], or to prepare fibers by electrospinning from hexafluoroisopropanol solutions [138] and [139].

Films composed of blends of *B. mori* fibroin and either chitin [140] or carboxymethyl chitin [141] were prepared by casting from aqueous solution, followed by treatment with aqueous methanol (inducing β -sheet assembly in the fibroin), and vacuum drying; yielding films in which the polymers were homogeneously distributed and interact via hydrogen bonding.

It is possible to deposit a layer of *B. mori* fibroin on porous β -chitin fibers by dip coating the β -chitin fibers in an aqueous solution of *B. mori* fibroin followed by drying and treatment

with aqueous methanol (inducing β -sheet assembly in the fibroin) [142] and [143]. Such chitin fibers can also be used to reinforce *B. mori* fibroin foams prepared by freeze-drying suspensions of the chitin fibers in fibroin hydrogels [144].

2.2.2.5. Chitosan

Chitosan is a linear polysaccharide produced by deacetylation of chitin, and is utilized in agriculture, biomedicine and filtration technologies. Fibers composed of *B. mori* fibroin and chitosan were electrospun from formic acid solution, immersed in aqueous methanol (to induce β -sheet assembly in the fibroin) and then vacuum dried. When the chitosan content was below 30 wt% the polymers were homogeneously distributed and the fibers were smooth with diameters of 130–780 nm. However, in fibers with higher chitosan content there was evidence of phase separation with chitosan-rich beads on the surface of the fibers [145].

Films composed of blends of *B. mori* fibroin and chitosan were prepared by casting from aqueous acetic acid solution, followed by air drying [146]. At all blend ratios the polymers were homogeneously distributed and the fibroin was β -sheet rich. Furthermore, a chitosan content of 30 wt% significantly improved the mechanical properties (tensile strength and initial tensile modulus) of the films relative to films cast from fibroin alone [147]. By contrast, films composed of *A. pernyi* fibroin and chitosan exhibited phase separation in films with chitosan contents >40 wt%, with chitosan particles dispersed in a fibroin-rich matrix [148].

Hydrogels composed of *B. mori* fibroin and chitosan were prepared simply by mixing acidified (pH 4) aqueous solutions of the polymers. The fibroin hydrogels interacted with the chitosan via both physical entanglement and hydrogen bonding interactions, and the viscosity of the hydrogels was found to increase as the concentration of chitosan increased [67]. Foams composed of *B. mori* fibroin and chitosan were prepared by freeze-drying aqueous solutions of the polymers. The foams were subsequently treated with mildly basic aqueous methanol (inducing β -sheet assembly in the fibroin and neutralizing the chitosan), and washed with basic water and then phosphate buffered saline solution. The mechanical properties (tensile strength and elastic modulus) increased with increasing fibroin content (as the gel was more highly cross-linked by β -sheets), and consequently their swellability in water was observed to decrease [149] and [150].

2.2.2.6. Hyaluronic acid

Hyaluronic acid is a glycosaminoglycan that is found in the extracellular matrix, and has been investigated for cosmetic and medical applications. Foams composed of *B. mori* fibroin and hyaluronic acid were prepared by freeze-drying aqueous solutions of the polymers. The foams were subsequently treated with aqueous methanol (inducing β -sheet assembly in the fibroin) and air-dried. The foams were demonstrated to be phase separated with a hyaluronic acid rich phase and a fibroin/hyaluronic acid phase. The breaking strength moderately increased at the expense of compressive modulus with increasing hyaluronic acid content [151], [152], [153] and [154]. It is possible to generate materials mimicking the natural extracellular matrix by incorporation of collagen coated *B. mori* fibroin fibers within analogous foams including chondroitin-6-sulfate (which is also found in the extracellular matrix) [155].

2.3. Man-made composites based on silk proteins and inorganic particles

2.3.1. Man-made composites based on silk proteins and metal nanoparticles

A focus of intense current research interest is the immobilization of biomolecules on nanoscale/colloidal substrates owing to their exciting applications in biochemistry, biotechnology and medicine. Many proteins can be conjugated to metal colloids via simply mixing the proteins with a metal colloidal sol (formed prior to mixing), and the protein typically binds the metal via amines or thiols displayed on their backbones. [Table 4](#) highlights a small selection of interesting examples.

Table 4.

Examples of man-made composite materials based on silk proteins and inorganic particles.

Morphologies	Components	Improvement vs. silk	References
Fibers or films	<i>B. mori</i> fibroin and silver nanoparticles	Antibacterial properties	[156] , [157] , [158] and [159]
Fibers	<i>B. mori</i> fibroin and gold nanoparticles	Electrical conductivity	[69] , [160] , [161] and [162]
Fibers	<i>B. mori</i> fibroin and magnetite nanoparticles	Response to magnetic fields	[69]
Various	<i>B. mori</i> fibroin and either A) calcium carbonate or B) calcium phosphate or C) silica	Biom mineralization (improved mechanical properties) needed for tissue engineering	[79] , [80] , [118] , [182] , [184] , [187] , [193] , [195] and [207]

[Table options](#)

2.3.1.1. Silver nanoparticles

Silver nanoparticles have interesting antibacterial and electronic properties, and are currently the subject of much debate due to potential environmental risks. *B. mori* fibroin fibers (either naturally spun or electrospun) have been coated with silver nanoparticles by (1) simply immersing the fibers in a warm aqueous solution of the nanoparticles [\[156\]](#) or (2) the tyrosine mediated reduction of Ag(I) to Ag(0) under basic conditions [\[157\]](#) and [\[158\]](#), or (3) the photochemical reduction of Ag(I) to Ag(0) (under irradiation with UV light) ([Table 4](#)) [\[159\]](#). The nanoparticles were shown to interact with some nitrogen and oxygen atoms present in fibroins.

2.3.1.2. Gold nanoparticles

Gold nanoparticles have interesting electronic and optical properties and are utilized widely in biological and biomedical applications. Solutions of *B. mori* fibroin have been used as an active scaffold for growing gold nanoparticles stabilized by a fibroin shell via an in-situ redox reaction between the tyrosine residues on the silk protein and Au(III) (which is reduced to Au(0)) during which the pH 9 solution changed from yellow to purple ([Table 4](#)). The gold cores were ca. 15 nm in size and the core/shell particles (of ca. 45 nm) were stable for months at room temperature [\[160\]](#) and [\[161\]](#). Naturally spun *P. phalangoides* spider silk fibers have

been coated in gold nanoparticles via incubation of the fiber in aqueous chloroauric acid, followed by washing with water, and drying ([Table 4](#)) [\[162\]](#). It is also possible to dip-coat natural spider silk fibers with hydrophobically functionalized gold nanoparticles with almost no detrimental effect upon the mechanical properties of the fibers [\[69\]](#).

2.3.1.3. Transition metal oxides/sulfides

Transition metal oxides and sulfides have a vast number of applications owing to their interesting electronic, magnetic and optical properties. Magnetic nanoparticles (such as superparamagnetic magnetite (Fe_3O_4)) have been used in numerous biomedical and technical applications, and semiconducting cadmium sulfide nanoparticles are utilized in photoresistors and solar cells. It has proven possible to dip-coat natural spider silk fibers with such nanoparticles with almost no detrimental effect to their mechanical properties ([Table 4](#)) [\[69\]](#).

Titanium dioxide is used as a white pigment in foods, paints and sunscreens. Films composed of *B. mori* fibroin and titanium dioxide nanoparticles (of ca. 80 nm) were prepared by air drying aqueous ethanol solutions that had been sonicated for 30 min. The titanium dioxide nanoparticles were evenly dispersed within a fibroin matrix that was β -sheet rich due to exposure to ethanol and sonication, and various techniques demonstrated the interaction of the nanoparticles with nitrogen and oxygen atoms displayed upon the backbone of the protein. The mechanical properties (maximum load, maximum stress and elasticity) of the fibroin films were improved by including titanium dioxide nanoparticles, and found to be optimal when the titanium dioxide content was between 0.2 and 0.4 wt% [\[163\]](#), [\[164\]](#) and [\[165\]](#).

2.3.2. Man-made composites based on silk proteins and biominerals

Biominerals are natural composite materials based upon biomolecules (such as proteins) and minerals produced by living organisms via processes known as biomineralization, yielding materials with impressive mechanical properties such as bones, shells and teeth. During biomineralization the biomolecule acts as a template for the formation of the mineral phase (sometimes even controlling which polymorph is produced) and such natural composite materials have been mimicked *in vitro* utilizing silk proteins as templates for the biomineralization process ([Table 4](#) shows a selection of interesting examples).

2.3.2.1. Calcium carbonate

Calcium carbonate is found in the shells of various marine organisms which are natural protein-based biomineral composite materials; mollusc shells are a particularly pertinent example composed of chitin, a silk-like protein, and calcium carbonate [\[166\]](#), [\[167\]](#) and [\[168\]](#). *In vitro* studies utilizing polystyrene substrates coated with *B. mori* fibroin were washed in a calcium chloride solution, followed by incubation in a dessicator containing ammonium carbonate (which slowly releases carbon dioxide). The liberated carbon dioxide reacts with the calcium ions complexed to the carboxylic acids displayed on the surface of the substrate, resulting in the formation of rhombohedral calcite crystals and a small number of vaterite crystals [\[142\]](#). The presence of magnesium ions in the solution used to wash the fibroin film was observed to promote the formation of aragonite crystals [\[169\]](#), as was the presence of β -sheets in the fibroin film [\[170\]](#). Interestingly, biomineralization of naturally spun *P. phalangoides* spider silk fibers in the same fashion yielded only calcite crystals [\[171\]](#).

Biom mineralization of porous β -chitin fibers coated with *B. mori* fibroin and macromolecules extracted from calcitic biominerals yielded calcite, as did control samples without the fibroin. In contrast, biomineralization of porous β -chitin fibers coated with *B. mori* fibroin and macromolecules extracted from aragonitic biominerals yielded aragonite, whereas control samples without the fibroin yielded vaterite, suggesting that the silk protein was involved in specific polymorph determination [142].

Particles composed of *B. mori* fibroin and calcium carbonate were prepared by precipitation from basic aqueous solutions of fibroin micelles, calcium chloride and sodium bicarbonate, in which the fibroin acted as a scaffold for the nucleation and crystallization of calcium carbonate microparticles via hydrogen bonding interactions. In the absence of the fibroin, a mixture of plate-like vaterite crystals (majority) and rhombic calcite crystals (minority) are formed [172]; in the presence of fibroin at pH 8.6, a mixture of spherical and rice-like crystals are formed (composed solely of calcite) probably because complexes of fibroin and CaCO_3 clusters are thermodynamically stable; whereas at pH 9.6, spherical crystals are formed (composed of a mixture of calcite and vaterite) due to the lower binding strength of complexes of fibroin and CaCO_3 [173], [174] and [175]. Interestingly, incubation of degummed naturally spun *B. mori* fibroin fibers in a solution at pH 8.6 led to the deposition of aragonite crystals on the fibroin template, that were aligned with the long axis of the fibers [176].

2.3.2.2. Calcium phosphate

Calcium phosphate is found in bone and tooth enamel which are both natural biomineral composite materials (protein/calcium phosphate). *In vitro* incubation of either silk proteins in solution (e.g. *B. mori* fibroin [177] or of *B. mori* fibroin and collagen [102]) or various water insoluble (i.e. β -sheet rich) silkworm/spider silk morphologies (films [178] and [179], hydrogels [180] and foams [181] and [182]) in simulated body fluids led to the deposition of hydroxyapatite nanoparticles on the surfaces of the silk scaffolds. Interestingly, incubation of β -sheet rich films of a genetically engineered chimeric protein based upon a major ampullate spidroin (from *N. clavipes*) and dentin matrix protein 1 in simulated body fluid led to the growth of hydroxyapatite crystals on the surface of the film, whereas films cast from the control protein without the dentin matrix protein 1 domain did not induce biomineralization [183].

Analogously it has proven possible to biomineralize silkworm/spider silk scaffolds with hydroxyapatite by repetitive cycles of: incubation in calcium rich solutions, washing with water, incubation in phosphate rich solutions, and washing with water (and minor variations of this procedure); as was demonstrated for fibers [184] and [185], films [186] and [187], particles [188] and [189] and foams composed of silk alone [187] or mixtures of a silk protein and poly(aspartic acid) [78], [79] and [80]. Interestingly, when naturally spun *P. phalangoides* spider silk fibers were biomineralized with hydroxyapatite, the *c*-axis of the crystals of hydroxyapatite were aligned with the long axis of the fiber due to interactions between the hydroxyapatite crystals and the proteins aligned with the long axis of the fiber by elongational flow during the natural spinning process [190].

An alternative approach is the generation of silk/hydroxyapatite composites using preformed hydroxyapatite nanoparticles. Examples include: hydrogels formed from mixtures of *B. mori* fibroin and hydroxyapatite nanoparticles [191]; and fibers composed of mixtures of *B. mori* fibroin, poly(ethylene oxide) and hydroxyapatite nanoparticles that were electrospun from

solution in dilute aqueous phosphate buffer (in order to minimize aggregation of the nanoparticles), immersed in methanol (rendering the fibers insoluble in water due to β -sheet assembly in the fibroin), then dried, yielding slightly rough fibers with diameters of approximately 510 nm. The polymers in the fibers were phase separated, and there was evidence of both aggregates of unaligned nanoparticles and well-dispersed nanoparticles that were aligned with the long axis of the fiber [118]. Other interesting examples utilize naturally spun silk fibers that were chemically modified with either poly(γ -methacryloxypropyl trimethoxysilane) [192] and [193] or poly(4-methacryloyloxyethyl trimellitate anhydride) [194] to which hydroxyapatite nanoparticles can subsequently be grafted (covalently or ionically respectively). The resulting composite fibers could be purified simply by sonication, washing in water, and finally freeze-drying; the hydroxyapatite nanoparticles were homogeneously distributed on the fibers and stable to extensive washing.

2.3.2.3. Silica

Silica is an amorphous biomineral found in the cell walls of Diatoms. Silaffins are low molecular weight proteins involved in the formation of protein/silica composites in nature. *In vitro* studies using a peptide derived from the repetitive motif found in silaffin proteins (known as the R5 peptide) demonstrated that this peptide promotes and regulates silica formation at neutral pH. Genetically engineered chimeric proteins based upon major ampullate spidroin (from *N. clavipes*) and R5 peptide are capable of both self-assembly and biomineralization. Fibers and films (electrospun/cast from HFIP solutions respectively) were treated with methanol to induce β -sheet formation in the major ampullate domain of the chimeric protein, and their subsequent incubation with a water-soluble silicon species led to biomineralization of silica [195].

A simple alternative to create silk/silica composites is to coat silk-based material templates with silica precursors (such as tetraethylorthosilicate (TEOS)) and subsequently heat them at 105 °C for several hours, as was demonstrated with major ampullate silk fibers of *N. madagascariensis* spiders [196] and cocoon fibers of *B. mori* fibroin silkworms [197]. Furthermore, the silk template can subsequently be removed by calcination at 600 °C, yielding a porous material in which the pore structure is determined by the silk-based material.

3. Applications of composite materials based on silk proteins

Nature does not have a monopoly on the creative use of composite materials, and in the final section of the review we will highlight some applications of man-made composite materials based on silk proteins.

3.1. Textiles

B. mori silk fibers have been used for centuries in the textiles industry due to their characteristic strength, moisture absorbance and luster; yet the natural fibers have shortcomings as they are prone to photoyellowing, and have poor rub resistance and wrinkle recovery. These shortcomings have been addressed by chemical modification of the naturally spun silk fibers [32], [33], [198], [199], [200] and [201] and by spinning new fibers

composed of regenerated silk proteins (isolated from the natural fibers) and other components.

3.1.1. Composites based on silk proteins and synthetic polymers

Fibers composed of poly(acrylonitrile) and its copolymers are cheap, antibacterial and stable to photo-oxidation, and are commonly used for textiles despite their poor water absorption and tendency to collect static electricity. Mixing *B. mori* fibroin with poly(acrylonitrile) notably improved the fibers water absorption and reduced their tendency to collect static electricity [36] and [52]. The mechanical properties of such fibers were acceptable for textile applications [202]. The addition of 2 wt% of a copolymer compatibiliser bearing on average two short poly(acrylonitrile) chains (of ca. 65 kDa) improved the tensile strength by 50% (relative to the fiber in the absence of the compatibiliser) whilst maintaining a fibroin-rich sheath that is important for good luster and handle [53] and [54].

3.1.2. Composites based on silk proteins and biopolymers

Cellulose fibers are widely used in the textile industry (in the form of cotton, hemp or jute) as it is cheap, biodegradable and moisture absorbent. Fibers composed of 30 wt% *B. mori* fibroin and 70 wt% cellulose had improved elastic moduli at the expense of breaking strength and yield stress relative to equivalent fibers produced solely from cellulose [131]. Chitin from crab and shrimp shells are readily available waste materials from food processing companies. Wet-spun fibers are relatively elastic yet weak, their breaking strength could be slightly improved by addition of 6 wt% *B. mori* fibroin allowing the manufacture of socks [137].

3.1.3. Composites based on silk proteins and metal nanoparticles

Silver ions are well-known to be toxic to microorganisms, and *B. mori* fibroin coated with silver nanoparticles was demonstrated to prevent the growth of the gram-positive bacterium *Staphylococcus aureus* on the surface of the silk fabric for up to five wash cycles, and inhibit the growth even after 10 wash cycles [156].

3.2. Sutures and dressings for wounds

Natural silk fibers have been used as sutures for wounds for centuries due to their strength, biocompatibility and low immunogenicity. In analogy to textiles, the mechanical properties of fibers determine if they can be used as sutures for wounds.

3.2.1. Sutures

Fibers composed solely of *B. mori* fibroin prepared by wet-spinning formic acid solutions into methanol had reasonable breaking strength, but were brittle, inflexible and had low knot strength (a feature of importance for the application of such fibers as sutures for wounds). Adding up to 50 wt% of poly(vinyl alcohol) was shown to increase the tenacity and elongation at break of the fibers (at the expense of the breaking strength) and the knot strength was observed to increase to ca. three times that of the fibers without poly(vinyl alcohol), which is sufficiently high for application as sutures for wounds [71]. Furthermore, silver nanoparticle coated *B. mori* fibroin fibers may be useful antimicrobial sutures [156].

3.2.2. Wound dressings

Non-woven mats prepared via electrospinning are currently being investigated for application as wound dressings, and non-woven mats of *B. mori* fibroin fibers coated with silver nanoparticles may be useful as antibacterial wound dressings [159]. Interestingly, *B. mori* fibroin films containing titanium dioxide nanoparticles were demonstrated to inhibit the growth of *Escherichia coli*, *P. aeruginosa* and *S. aureus* [165].

Hydrogels composed of *B. mori* fibroin and cross-linked poly(ethylene oxide) were demonstrated to be non-cytotoxic in *in vitro* studies using mouse fibroblast (L929) cells, and were demonstrated to promote wound healing (relative to a Vaseline gauze control) *in vivo* in rats [64], [65] and [66]. Similarly, *B. mori* fibroin hydrogels containing hydroxyapatite nanoparticles were demonstrated to promote wound healing *in vivo* in pigs [191].

3.3. Tissue scaffolds

Natural extracellular matrices are predominantly composed of nanofibrous collagen and proteoglycans (such as chondroitin sulfates and hyaluronic acid), however the widespread therapeutic use (e.g. for replacing/restoring malfunctioning tissues) of collagen and proteoglycans in the clinic is limited due to their cost – an attractive alternative is the use of combinations of cheaper bio/synthetic polymers.

3.3.1. Composites based on silk proteins and other proteins

Electrospun fibers composed of *B. mori* fibroin and type I calf skin collagen that were cross-linked with glutaraldehyde, were demonstrated to support cell adhesion and proliferation of both human epidermal keratinocytes (NHEK) and fibroblasts (NHEF) cells *in vitro*. Interestingly, electrospun fibers composed of unmixed *B. mori* fibroin and type I calf skin collagen (that were prepared by electrospinning from two syringes simultaneously and subsequently cross-linked with glutaraldehyde) showed much higher levels of cell adhesion and proliferation for NHEK cells *in vitro* [95]. Foams composed of *B. mori* fibroin and either type I bovine collagen or recombinantly produced human-like collagen were demonstrated to support cell adhesion and proliferation of human hepatocellular carcinoma (HepG2) cells *in vitro*, with significant improvements in both adhesion and proliferation in comparison to films composed of *B. mori* fibroin alone [101], [103] and [203].

Embedding collagen coated *B. mori* fibroin fibers within foams composed of mixtures of collagen, hyaluronic acid and chondroitin-6-sulfate, yields materials that were shown to support cell adhesion and proliferation of human anterior cruciate ligament cells *in vitro* at levels markedly improved from collagen coated *B. mori* fibroin fibers alone. *In vivo* studies of these composites implanted in dogs showed the formation of new blood vessels and minimal inflammatory reaction after 6 weeks [155].

In vitro studies performed on foams composed of *B. mori* fibroin and hyaluronic acid showed improved adhesion and proliferation of human mesenchymal stem cells [154] and primary neural cells (cortical neurons from rat embryos) [153] in comparison to either of the constituent polymers alone.

3.3.2. Composites based on silk proteins and polysaccharides

Composite materials (in which microspheres composed of alginate and *A. mylitta* fibroin were embedded in *A. mylitta* fibroin foams) were shown to support the adhesion and

proliferation of AH927 fibroblast cells *in vitro* [130]. Interestingly, the differentiation of human mesenchymal stem cells *in vitro* was demonstrated to be determined by the growth factor released from *B. mori* fibroin microspheres (containing either osteogenic bone morphogenic protein-2 or chondrogenic insulin-like growth factor) dispersed in alginate hydrogels [119].

Electrospun fibers composed of *B. mori* fibroin and chitin were demonstrated to support cell adhesion and proliferation of both human epidermal keratinocytes (NHEK) and fibroblasts (NHEF) cells *in vitro* [138]. Interestingly, electrospun fibers composed of unmixed *B. mori* fibroin and chitin (that were prepared by electrospinning from two syringes simultaneously) showed much higher levels of cell adhesion and proliferation for NHEK cells *in vitro* [139]. Foams composed of *B. mori* fibroin and chitosan were shown to support cell adhesion and proliferation of mouse fibroblast (L929) cells *in vitro* [144], and *in vivo* studies show that after 4 weeks of implantation in the abdominal wall of guinea pigs, the implants had integrated seamlessly being partially degraded and remodeled [150]. More recently, such scaffolds have been implanted in the bones of sheep, after up to 12 weeks *in vivo* the implants were shown to be partially degraded and remodeled, thereby demonstrating their potential for use in the clinic [204].

3.3.3. Composites based on silk proteins and synthetic polymers

Poly(l-lactide) is a popular polymer for biomedical applications due to its biodegradability. Electrospun fibers composed of *Agelena labyrinthica* spidroin and poly(d,l-lactide) were demonstrated to support cell adhesion and proliferation of African green monkey kidney [CCL81 Vero] cells *in vitro* [93]. Electrospun fibers with a core-shell morphology in which the core was composed of *B. mori* fibroin and gelatin and the surface was poly(l-lactide) were demonstrated to support cell adhesion and proliferation of 3T3 mouse fibroblasts and human umbilical vein endothelial cells *in vitro*. Tubes formed of such fibers are currently being investigated for application as artificial blood vessels due to their biocompatibility, mechanical properties and porosity [205] and [206].

Composite materials in which a *B. mori* fibroin foam filled the pores of a poly(ϵ -caprolactone) foam were shown to support cell adhesion and proliferation of human fibroblast (ATCC HFL-1) cells *in vitro* better than foams composed solely of poly(ϵ -caprolactone) [85].

Foams composed of *B. mori* fibroin and poly(l-lactide) were demonstrated to support cell adhesion and proliferation of both mouse macrophage (RAW 264.7) and human hepatocellular carcinoma (HepG2) cells *in vitro* better than foams composed of poly(l-lactide) alone. Moreover, the presence of *B. mori* fibroin reduced the inflammatory reaction of the macrophages [92].

Foams composed of *B. mori* fibroin and poly(aspartic acid) supported the adhesion and proliferation of human mesenchymal stem cells *in vitro*, and osteogenic differentiation was induced by inclusion of bone morphogenic protein-2 within the foam and culture medium; such foams may find application for replacement of broken bones [78].

3.3.4. Composites based on silk proteins and inorganic particles

Electrospun fibers composed of *B. mori* fibroin and poly(ethylene oxide) supported the adhesion and proliferation of human mesenchymal stem cells *in vitro*, however levels of differentiation towards osteogenic outcomes were low despite the osteogenic media. Incorporation of bone morphogenic protein-2 into the spinning dope notably improved differentiation of the stem cells towards osteogenic outcomes. Furthermore, addition of hydroxyapatite nanoparticles to the spinning dope yielded fibers with the nanoparticles embedded inside and was found to improve bone formation [118] and [207]. Subsequent studies demonstrated that coating *B. mori* fibroin fibers, films or foams with hydroxyapatite nanoparticles improved the adhesion and proliferation of mouse fibroblast (L929) cells [193], mouse pre-osteoblastic (MC3T3-E1) cells [187], rat osteoblast cells [184], bone marrow stromal cells [79] and [80], human mesenchymal stem cells [187] and human osteoblast (MG-63) cells *in vitro* [182]. Recent *in vivo* studies have also shown that hydroxyapatite biomaterialized foams composed of *B. mori* fibroin and poly(aspartic acid) have great potential for clinical application for bone regeneration [79] and [80].

3.4. Biocompatible coatings

Silk films are promising candidates for biocompatible coatings for biomedical implants owing to the facility with which silk proteins can be processed into films with differing surface properties (morphology, hydrophilicity etc...), their biodegradability and low levels of immunogenicity *in vitro/in vivo*. Coating biomedical implants with silk films potentially imbues their surfaces with anticoagulant properties, or inhibits/promotes cell adhesion [208].

3.4.1. Anticoagulant coatings

Water-insoluble anticoagulants are important coatings for stents, and water-insoluble films composed of *B. mori* fibroin and carboxymethyl keratin [122] or poly(acrylic acid) decorated with tetramethylpyrazine [209] were demonstrated to be more antithrombogenic than either of the biopolymers alone in *in vitro* blood clotting tests.

Interestingly, it is also possible to control the release of heparin (a natural anticoagulant) from films composed of pellethane and *B. mori* fibroin particles. The release profile of heparin could be controlled by variations in heparin loading, film thickness (thicker films released heparin over longer periods of time), and the ratio of pellethane to fibroin (greater fibroin content leads to slower release, probably due to hydrogen bonding interactions between the fibroin and heparin) [94].

3.4.2. Antibacterial coatings

As discussed above in Section 3.1.3, antibacterial coatings are of great industrial importance, and coating both poly(propylene) films and poly(amide) foams with *B. mori* fibroin was demonstrated to inhibit the adhesion of *Staphylococcus epidermis* gram-positive bacteria cells *in vitro* [210]. Additionally, composite materials based upon silk proteins and inorganic nanoparticles (e.g. silver nanoparticles [159]) or titanium dioxide nanoparticles have been demonstrated to inhibit the growth of *E. coli*, *P. aeruginosa* and *S. aureus* [165].

3.4.3. Improved cell adhesion

The biocompatibility of materials to be implanted in people (such as hip replacements) are sometimes rejected from the body, and biocompatible coatings such as silks are one method of improving their acceptance.

Coating various poly(urethane) films and foams [87], [88] and [89] with *B. mori* fibroin was demonstrated to improve cell adhesion and proliferation of human fibroblast cells *in vitro* relative to the uncoated materials. Similarly, poly(propylene) films and poly(amide) foams coated with *B. mori* fibroin were demonstrated to support cell adhesion and proliferation of mouse fibroblast (L929) cells *in vitro* [210].

Poly(allylamine) is a polycationic polymer that is highly soluble in water that it utilized widely in biomedical applications. Films composed of *B. mori* fibroin and poly(allylamine) were found to be more stable in water than films composed only of poly(allylamine), and such films were demonstrated to support cell adhesion and proliferation of both insect (*A. pernyi* and *B. mori* silkworm) and mammalian (mouse fibroblast L929) cells *in vitro* better than either of the constituent polymers alone [56].

The adhesion of mouse fibroblast L929 cells to films composed of *B. mori* fibroin and poly(lactic acid) were shown to be better than either of the constituent polymers alone [91].

Mimicry of the extracellular matrix is a popular approach to promote cell adhesion, and with this goal in mind numerous materials have been prepared that incorporate components of the extracellular matrix. Films composed of *B. mori* fibroin and rat-tail collagen were demonstrated to support cell adhesion and proliferation of rat liver cells *in vitro* [99]. Films composed of *B. mori* fibroin and either type I bovine collagen or recombinantly produced human-like collagen were demonstrated to support cell adhesion and proliferation of human hepatocellular carcinoma (HepG2) cells *in vitro*, with significant improvements in both adhesion and proliferation in comparison to films composed of *B. mori* fibroin alone [97] and [98]. Films composed of *B. mori* fibroin and recombinantly produced proteins based upon *N. clavipes* spider dragline silk (incorporating the RGD integrin recognition sequence) was shown to support cell adhesion and proliferation of mouse osteoblast (MC3T3-E1) cells *in vitro* [111].

3.5. Drug delivery

A reliable and controllable release profile is important for a drug to have its optimal effect and minimize side effects [211] and [212]. Composite materials based on silk proteins are attractive drug carriers due to their biocompatibility and highly tunable morphologies.

3.5.1. Low molecular weight drugs

Films composed of *B. mori* fibroin and chitosan (cross-linked with glutaraldehyde) and various low molecular weight drugs (ampicilline trihydrate, dichlofenac sodium, salicylic acid and theophylline) released most of their payload within 30 min of incubation in various pH buffered solutions *in vitro* [213]. Films composed of *B. mori* fibroin and between 12.5 and 25 wt% poly(ethylene oxide) were used to coat tablets of theophylline. Uncoated tablets dissolved completely within 210 min, whereas dissolution of the coated tablets was not complete until 300 min and displayed zero order kinetics *in vitro* [214].

Hydrogels composed of either a poly(ethylene oxide)-poly(propylene oxide) copolymer (Pluronic F-127) or chitosan with *B. mori* fibroin were shown to release buprenorphine more slowly than hydrogels composed of fibroin alone *in vitro* [67].

Adenosine is a potential therapeutic for epilepsy which is a major health problem for many people. A complex *B. mori* fibroin composite material comprising: fibroin/adenosine microspheres coated in silk, embedded in a fibroin foam that is filled with fibroin/adenosine solution, and subsequently coated in a multilayer film (composed of alternating layers of fibroin and adenosine) was demonstrated to control the release of the drug over a period of days *in vitro* and *in vivo* when implanted in rats brains [215].

3.5.2. High molecular weight drugs

Films composed of *B. mori* fibroin and various enzymes [104], [105], [106], [107] and [108] have been shown to retain their catalytic activity for many days with minimal leaching of the enzyme *in vitro*. Microspheres composed of a model enzyme drug and either alginate or poly(lactide-co-glycolide) were coated with *B. mori* fibroin, the silk coating slowed enzyme release from the order of ten's of hours to ten's of days *in vitro* [90]. Growth factors or enzymes trapped within *B. mori* fibroin microspheres and subsequently dispersed in either fibroin foams or alginate hydrogels were demonstrated to be released over a period of days *in vitro* [119]. Likewise, high molecular weight drugs encapsulated in microspheres composed of alginate and *A. mylitta* fibroin suspended in *B. mori* fibroin foams were demonstrated to be released without initial bursts for 35 days [130].

Films composed of *B. mori* fibroin and hyaluronic acid were demonstrated to be responsive to both pH and electrical stimuli. At low pH values (between pH 2 and 3) fibroin and hyaluronic acid form polyion complexes which dissociate at neutral pH, hence the β -sheet cross-linked films were observed to be more water permeable at neutral pH (yet still mechanically stable); the complexes are also responsive to electrical stimuli, and application of a potential difference led to dissociation, and consequent swelling of the films. *In vitro* studies showed that when swollen the films were permeable to Timolol maleate which is well tolerated in humans upon transdermal application, and suggests that such films may find application in membrane-permeation controlled formulation [216] and [217].

3.6. Materials with novel electronic, magnetic and optical properties

Coating naturally spun major ampullate silk fibers with poly(pyrrole) or gold nanoparticles (electrical conductors) or cadmium sulfide particles (a semiconductor) potentially imparts novel electronic properties to the fibers; likewise fibers coated with magnetite nanoparticles yields fibers that respond to magnetic fields which have potential application in audio reproduction [69] and [162]. Electrospun fibers composed of *B. mori* fibroin that were coated with a layer of carbon nanotubes were demonstrated to have markedly improved electrical conductivity (ca. 10^{-4} S cm⁻¹) in relation to electrospun fibers composed solely of *B. mori* fibroin (ca. 10^{-15} S cm⁻¹) [45]. *B. mori* fibroin microspheres coated with a layer of carbon nanotubes were demonstrated to have similar electrical conductivities (ca. 10^{-4} S cm⁻¹) [46]. Such materials may find application in textiles as they would reduce the tendency of the fabrics to collect static electricity. Moreover, it is also possible to prepare materials (e.g. films) with interesting nonlinear optical properties by incorporation of green fluorescent protein within the silk matrix [117].

3.7. Enzymatically active biomaterials

One method of preparing biosensors is the modification of the surface of electrodes with enzymes (via covalent immobilization). Yet a problem commonly encountered with this approach is the denaturation of the enzyme due to non-covalent interactions with the electrode surface, which renders them catalytically inactive. One solution to this problem is to embed the enzyme in a protein matrix which helps to maintain the enzymes native structure (thereby retaining its catalytic activity) [104], [105], [106], [107] and [108]. An elegant example of this approach utilized films composed of the enzyme horseradish peroxidase and *B. mori* fibroin stabilized gold nanoparticles (further cross-linked by interprotein β -sheet formation). The enzyme maintained its catalytic activity and electrodes coated with such films responded swiftly to the reduction of hydrogen peroxide, demonstrating their applicability as biosensors [161]. Interestingly, we have also demonstrated it to be possible to covalently immobilize an enzyme (β -galactosidase) on a silk-based material with maintenance of the catalytic activity of the enzyme [218].

4. Conclusions

Natural composite fibers produced by silkworms and spiders have been used by humans for centuries. Human creativity has produced a variety of composite materials (including fibers, films, gels, foams and particulates [3] and [34]) based on silk proteins with a number of exciting applications. We envisage that in the future such composite materials will be of economic importance not only in textile applications but in high-tech applications such as tissue scaffolds and drug delivery devices; and moreover, that *de novo* designed silk-like proteins and polymers [28] will be of great importance due to their highly tunable structures.

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